PESTICIDE RESIDUES IN ANIMAL TISSUES

Supercritical Fluid Extraction of Poultry Tissues Containing Incurred Pesticide Residues

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A method using supercritical carbon dioxide to extract fat from poultry tissue was developed. Tissues used in this study were peritoneal fat, breast tissue, leg and thigh tissue, and liver from chickens fed rations containing heptachlor, dieldrin, and endrin. The fat was isolated from the peritoneal tissue by supercritical fluid extraction (SFE) and by thermal rendering. The fat was removed from the breast tissue, leg and thigh tissue, and liver by SFE and solvent extraction. The results indicate that recoveries of organochlorine pesticide from the peritoneal, breast, leg, and thigh tissues by SFE extraction are equivalent to those obtained by conventional extraction methods. The pesticide recoveries by SFE extraction of the liver were higher than those obtained by solvent extraction.

Analytical methods used to determine chlorinated pesticides in animal tissues have been applied to the isolated fatty material containing the incurred pesticide residues (1–6). Regulatory agencies and analytical laboratories currently use organic solvents to extract the fat from the animal tissue (6–7). Recently, there has been considerable concern about the health hazards associated with the use of organic solvents as well as the impact of their subsequent disposal on the environment (8). Supercritical fluid extraction (SFE) has been used to separate the lipid material containing pesticide residue from animal tissue (9) and fish tissue (10) as an alternative to solvent extraction. The present paper describes a method in which the fat from poultry (chickens) that have been fed 3 chlorinated pesticides has been extracted both by supercritical CO_2 and by conventional extraction methods. Quantitative recovery of the pesticides by the SFE extraction method is compared to the recovery by conventional solvent extraction techniques and by thermal rendering of fat.

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The reported method, which can be generically classified as "off-line" SFE, is preferred to an "on-line" SFE method (11), because the latter is difficult to accomplish in the presence of lipid coextractives. The described SFE technique has been applied successfully to relatively large fat and dehydrated tissue samples. The study is also unique in that SFE was performed on biological tissues that contained actual incurred residues, rather than fortified tissue.

Experimental

Apparatus

Gas chromatograph. — Hewlett-Packard GC 5890 equipped with ^{63}Ni electron capture detector (ECD) (Hewlett-Packard, Avondale, PA); 2 m × 4 mm id glass column (GP 1.5% SP-2250/1.95% SP-2401) (Supelco, Bellefonte, PA). Temperatures: oven 200°C, injector 220°C, and detector 350°C. Helium flow rate, 40 mL/min.

Pressure apparatus.—Haskel air-driven compressor (Burbank, CA); Autoclave micro-metering valve (Erie, PA).

Chemicals and Reagents

Petroleum ether and hexane were obtained from J.T. Baker, Inc. (Phillipsburg, NJ). Neutral alumina, Brockman Activity 1 (Fisher Scientific, Pittsburgh, PA) was heated to 800°C for 4 h, cooled, and activated by adding 5% distilled water by weight. Pesticide standards were purchased from Supelco. Carbon dioxide with a purity of 99.95% was obtained from National Welding Supply, Bloomington, IL.

Production of Incurred Residues

Chicken samples containing incurred chlorinated pesticide residues were generated by L. Rowe (College Station, TX). Nine chickens (22-month-old White Leghorn breeder hens) were fed diets containing 0.45 ppm each of heptachlor,

dieldrin, and endrin for 55 days to provide a concentration of 0.51 ppm of each pesticide (12); 5 control chickens were fed a pesticide-free diet. The chickens were sacrificed 2 days after withdrawal from the pesticide-treated feed. Previous feeding studies of chlorinated hydrocarbon pesticides had indicated that concentration of the residues reached a plateau after 6–7 weeks of feeding (12). The chicken tissue was removed from the bones and portioned into breast, leg/thigh, peritoneal fat, and liver tissue. The tissue samples were individually packaged, frozen, and sent to the National Center for Agricultural Utilization Research (NCAUR) in Peoria, IL. These samples were used in this study.

Extraction

Samples were prepared identically for both conventional fat separation techniques (13) and SFE. The peritoneal fatty tissue was ground with the aid of a Kitchen Aid food grinder and divided into 2 portions. One part was SFE-extracted to separate the fat, and the other part was thermally rendered at 80°C for 3 h to separate the fat.

The breast and leg/thigh muscle were ground and divided into 3 portions. Two parts were oven-dried at 50°C until the moisture content of the muscle was <5% by weight. One of the dried portions was extracted by SFE. The other portion was solvent-extracted by mixing it with 100 mL petroleum ether (PE) in a blender for 5 min; the solution was filtered, and the solvent was removed by evaporation on a steam bath. When ca 10 mL solvent was left, the remainder of the PE was removed by a nitrogen stream. The third portion of ground tissue was dried and extracted in 1 step by mixing PE and Na_2SO_4 in a blender; the solvent was decanted from the tissue, and the fat was recovered after solvent removal by using a steam bath and nitrogen flow as described previously.

The liver was ground, oven-dried at 50°C, and divided into 2 portions; 1 portion was extracted by SFE, and the other was extracted with PE in a blender. The PE extract was decanted from the liver tissue, and the PE was removed by evaporation on a steam bath followed by nitrogen drying.

The SFE extractions were performed in an extraction apparatus previously described (9). Two stainless steel extraction tubes (1.75 × 30.5 cm and 1.75 × 56 cm) were used, depending on the size of the sample. The peritoneal fat samples (25–30 g) were spread on glass wool supports, which were placed on plastic sheets (4 × 28 cm); the sheets were then rolled to fit into the extraction tubes (1.75 cm id). The extraction tubes (pressure rated at 10 000 psi) were then put into a converted GC oven, and the temperature was gradually raised to 80°C as the pressure was raised to 10 000 psi and maintained by the micrometering valve located before the collection flask. The flow rate of the CO_2 gas was measured at the exit port of the collection flask by an American dry test meter (Philadelphia, PA).

Extraction fluid flow rates for the peritonal fat ranged from 10 to 20 L/min, as measured under ambient conditions, and the extraction times varied from 30 to 40 min. The extracted fat, containing the incurred residues, was collected in glass round-bottom flasks. Ground meat tissue (ca 20–30 g) and liver samples (7–10 g) were placed directly in the extraction tubes. The same temperature and pressure were used to extract the ground meat and liver samples as were used for the peritoneal fat. The CO_2 flow rates were kept at 2–4 L/min for ca 2 h for the ground meat samples and 2 L/min for 45 min for the liver samples. Crude fat was determined on all tissues (7), and analysis indicated that >96% of the theoretical fat was removed by SFE extractions (9). SFE extractions of fat from control chickens were made to determine the optimum conditions for each tissue type.

Table 1. ECD/GC results for poultry tissues extracted with supercritical carbon dioxide[a]

		Pesticide, ppm in lipid extract		
Chicken No.	Tissue type	Heptachlor epoxide	Dieldrin	Endrin
1	Fat	0.88	2.93	2.32
1	Leg/thigh	0.91	2.82	2.24
1	Breast	1.04	3.06	2.34
2	Fat	0.80	2.57	2.41
2	Leg/thigh	0.91	2.53	2.15
2	Breast	1.09	2.21	1.75
3	Fat	0.96	2.73	2.08
3	Leg/thigh	0.82	2.30	1.75
3	Breast	1.42	2.68	1.98
4	Fat	1.12	3.00	2.32
4	Leg/thigh	1.08	2.86	2.22
4	Breast	1.56	3.08	2.24
CV, %[b]	Fat	6.1	4.1	3.7
CV, %	Leg/thigh	3.7	5.3	5.8
CV, %	Breast	3.9	3.8	3.5

[a] Differences determined by an analysis of variance.
[b] CV, coefficient of variation (av).

Cleanup and Analysis

The pesticide residues were separated from the fat and determined by Food Safety and Inspection Service method 5.002, a micro alumina column method for the separation of chlorinated hydrocarbons (13). For GC analysis, aldrin was added as an internal standard to the injection solution in isooctane, and a 2.0 µL aliquot was injected. Analyses were made in duplicate to determine repeatability.

Results and Discussion

Pesticide residue concentration was determined in lipid material extracted from the individual chicken tissues. From the 14 chickens sampled, the average weight % fat content of the peritoneal fatty tissue (F) was 88%; 24% fat was extracted from the leg/thigh (L/T) tissue, 5.8% from the breast (B), and 11% from the liver.

Pesticide residues were not found in any of the tissues of the 5 control chickens fed the pesticide-free diet. Pesticide concentrations in the 3 tissue types are shown in Table 1. Comparisons

Table 2. Analytical results for peritoneal fat processed by SFE or thermal rendering[a]

	Pesticide, ppm in lipid extract					
	Heptachlor epoxide		Dieldrin		Endrin	
Chicken No.	SFE[b]	REN[c]	SFE[b]	REN[c]	SFE[b]	REN[c]
1	0.88	0.97	2.93	2.91	2.39	2.39
2	0.80	0.79	2.57	2.43	2.41	2.26
3	0.96	1.08	2.73	3.15	2.08	2.57
4	1.12	0.83	3.00	2.73	2.32	2.40
5	0.86	0.96	2.92	3.09	2.32	2.40
6	0.70	0.74	2.38	2.46	2.06	2.23
7	0.56	0.62	2.33	2.30	2.00	2.11
8	0.59	0.57	1.97	1.97	1.95	1.93
9	0.66	0.60	2.34	2.19	2.38	2.17
CV, %[d]	4.2	3.7	5.0	2.0	3.3	2.3

[a] Comparison by paired *t*-tests found no differences between extraction methods.
[b] SFE, supercritical fluid extraction.
[c] REN, thermal rendering.
[d] CV, coefficient of variation (av).

were tested within an analysis of variance at $P < 0.05$. Heptachlor epoxide formed from metabolized heptachlor was higher in the breast muscle than in the peritoneal fat and leg/thigh tissue. There were also significant differences ($P < 0.05$) in the heptachlor epoxide residue between chickens. Chickens 1 and 4 had higher levels of dieldrin than chickens 2 and 3. There were no significant differences ($P < 0.05$) in the amounts of dieldrin or endrin residues present in the fat extract of the 3 tissue types.

The concentrations of the pesticides from the peritoneal fat extracted by SFE and from thermal rendering of the fat are compared in Table 2. A paired *t*-test comparing the 2 extraction techniques found no significant difference ($P < 0.05$) between the 2 methods for any of the 3 pesticides studied. Each value in Table 2 is the average of 2 determinations. At the bottom of the table is the average coefficient of variation (CV), showing the repeatability of the GC determinations.

Table 3 compares the data from the 3 extraction methods (PE, dehydration achieved by addition of Na_2SO_4 to PE and extracted; DPE, sample air-dried and extracted with petroleum ether; and SFE, supercritical fluid-extracted) for the breast and the leg/thigh tissues.

No significant differences ($P < 0.05$) were found by an analysis of variance for the PE vs DPE, PE vs SFE, or DPE vs SFE for any of the 3 pesticides. Chicken 7 generally tended to have lower levels of pesticide residues than the other 2 chick-

Table 3. Results from poultry tissue extracted by supercritical fluid vs solvent extraction methods[a]

		Pesticide, ppm in lipid extract								
		Heptachlor epoxide			Dieldrin			Endrin		
Chicken No.	Tissue type	PE[b]	DPE[c]	SFE[d]	PE	DPE	SFE	PE	DPE	SFE
6	Breast	0.80	0.80	0.86	2.79	2.77	2.79	2.42	2.34	2.39
6	Leg/thigh	0.71	0.76	0.80	2.61	2.80	2.71	2.30	2.41	2.36
7	Breast	0.52	0.48	0.46	2.09	2.02	2.08	1.70	1.58	1.70
7	Leg/thigh	0.52	0.52	0.49	2.00	1.96	1.80	1.72	1.80	1.65
9	Breast	0.67	0.70	1.01	2.40	2.35	2.04	2.32	2.29	2.13
9	Leg/thigh	0.58	0.61	0.74	2.20	2.26	2.22	2.13	2.15	2.10
CV, %[e]	Breast	3.7	3.0	1.4	4.3	7.3	3.7	4.0	7.5	3.8
CV, %	Leg/thigh	3.1	3.0	3.1	6.8	5.2	5.5	5.4	6.5	5.6

[a] Differences determined by an analysis of variance.
[b] PE, Na_2SO_4 added, extracted with petroleum ether.
[c] DHE, dehydrated, extracted with petroleum ether.
[d] SFE, supercritical fluid extraction.
[e] CV, coefficient of variation (av).

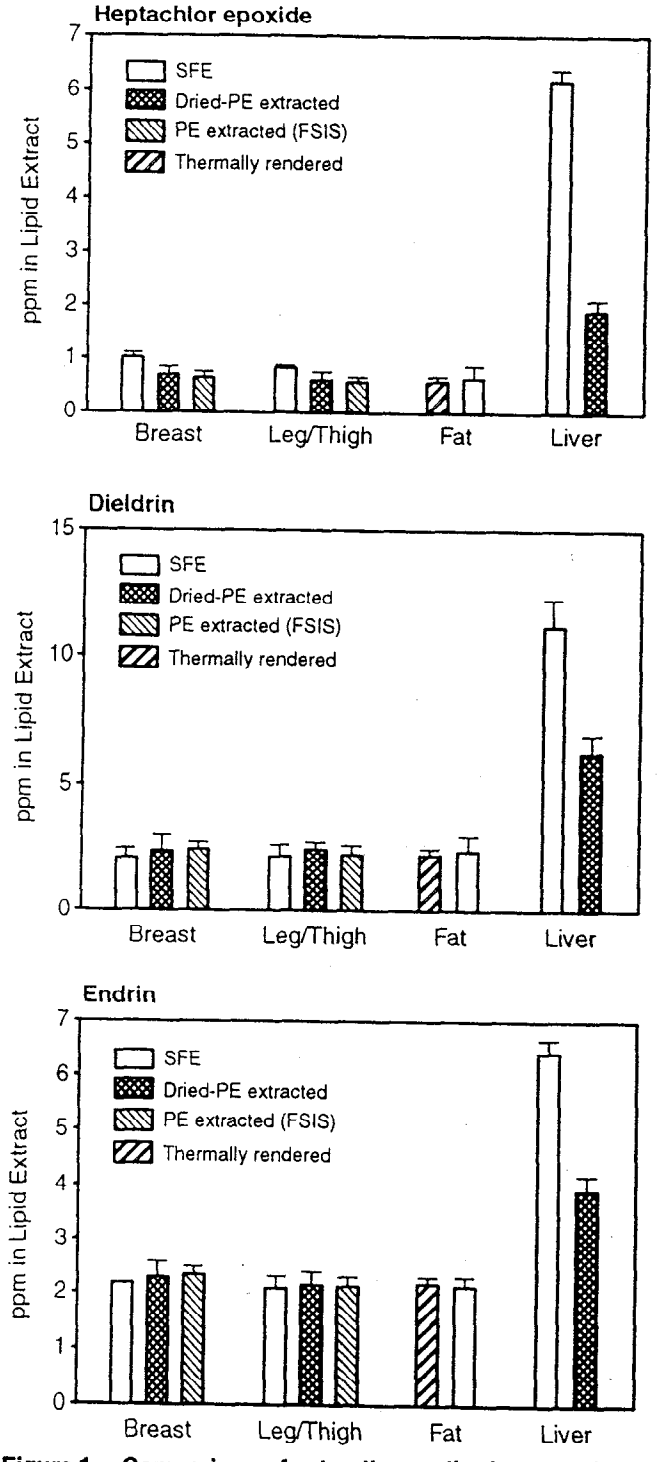

Figure 1. Comparison of extraction methods on poultry tissues from chicken No. 9. Standard deviation is indicated by the marks at the top of each bar.

ens studied. For these data, where a difference was found between tissues, higher levels of dieldrin were found by PE extraction and higher levels of endrin by SFE extraction for breast tissues than for leg/thigh tissues. The average CV of each extraction method for all analyses of the breast and leg/thigh samples was 4.6% for PE extraction, 5.4% for DPE extraction, and 3.8% for SFE extraction.

Pesticide residue levels from the fat extract of chicken 9 were plotted, and Figure 1 shows the concentrations determined by 4 methods of extraction of all the tissue types. The standard deviation for the duplicate GC determinations is shown at the top of each individual bar. The data trend for this chicken was representative of all of the chickens studied. Peritoneal fat extracted by the 2 methods of thermal rendering and SFE showed no difference in the pesticide concentrations. Also, there was no significant difference among the 3 extraction methods in the amount of pesticide found in the breast and leg/thigh tissues, as was also reported in the previous tables. However, the data in Figure 1 indicated that the amount of each pesticide residue found in the liver was greater by SFE than by solvent extraction. This result is possibly due to the superior mass transport properties exhibited by supercritical fluids vs conventional organic solvents (14), i.e., SC-CO_2 is able to penetrate the liver tissue more effectively and to extract the pesticides, and, thus, yielded higher analyte recoveries from all the livers that were extracted.

The incurred residues found in the poultry tissues were 2-6 times their concentration in the residue-inducing feed. This is in agreement with the results of Cummings et al. (12); however, the lower values found for heptachlor epoxide relative to dieldrin (15, 16) and endrin may be due to a difference in the rate of metabolic conversion of heptachlor to its epoxide within the chicken's body.

Overall, with the exception of the liver, the analytical data suggest that the distribution of the individual chlorinated pesticides in the fat from different types of hen tissue is relatively constant. This is consistent with the detoxifying role of the liver in the mammalian body, as reported by Groves et al. (17).

The results of this study indicate that supercritical fluid extraction is as effective as conventional thermal rendering or solvent extraction techniques. This research has the potential for establishing a "solventless" extraction method, eliminating the cost associated with hazardous solvent disposal, and avoiding the exposure of laboratory personnel to such chemical agents.

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